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Oxygen Uptake and Transport in the Lamellibranch Mollusc Modiolus demissus

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OXYGEN UPTAKE AND TRANSPORT IN THE LAMELLIBRANCH MOLLUSC *MODIOLUS DEMISSUS*

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ABSTRACT

The mechanisms of oxygen uptake and transport are examined in Modiolus demissus, a lamellibranch mollusc with no specialized oxygen carrier in its blood. Modiolus, which has a high ventilatory rate and a large gill surface area, equilibrates its blood with oxygen to a degree comparable to species with functional respiratory pigments. The high blood PO_2's suggest that gas exchange occurs at several superficial sites in addition to the gills. However, the small reduction of PO_2 in deep tissue indicates that, at high oxygen levels, the blood plays only a small role in oxygen transport. $\dot{V}O_2$ is 50% lower in air than in water, and during air exposure, there is almost complete equilibration between the PO_2 of water remaining in the mantle cavity and the blood. This relationship and the observation that aerobic respiration generally ceases when the water PO_2 falls to the levels which occur in the mantle cavity, suggest that aerobic metabolism persists in superficial tissues by means of direct uptake of oxygen from the air, without participation of the blood. Deep tissue is believed to carry on primarily anaerobic metabolism during air exposure.

OXYGEN UPTAKE AND TRANSPORT IN THE LAMELLIBRANCH MOLLUSC *MODIOLUS DEMISSUS*

INTRODUCTION

While specialized oxygen carriers in the blood are found in several classes of molluscs, they have been lost within the class Lamellibranchia. In the lamellibranchs, hemoglobin is found circulating in the blood among members of the primitive taxodont families Arcidae and Glycymeridae, and it has a scattered occurrence in a few more advanced families. However, hemoglobin is absent from the vast majority of species. In the arcid clam Noetia ponderosa, a minimum of 90% of the oxygen carried by the blood is bound to hemoglobin. In normoxic waters, the hemoglobin is fully oxygenated at the gill, and it delivers about 50% of its load to the tissues, which accounts for over half of total oxygen uptake (Freadman and Mangum, 1976; Deaton and Mangum, 1976). The remaining portion of the oxygen consumed is carried in the plasma or is taken up by tissues directly from water.

The loss of hemoglobin, which reduces the oxygen carrying capacity of the blood by an order of magnitude, raises the question of the respiratory role of the blood. Previous studies of molluscan respiration suggest that two adaptations mitigate the loss: 1) Gill surface areas are larger (about 2X) and 2) Ventilation rates are much greater (10-15X) (Deaton and Mangum, 1976). Rates of total oxygen consumption appear to be similar or even greater in several species without hemoglobin. However, it is not clear that these adaptations are able to compensate fully in maintaining the respiratory role of

the blood without a respiratory pigment. The only report of blood oxygen levels in the higher lamellibranchs was made by Taylor (1976b), who found high PO_2's in the heart of Arctica islandica, which has no hemoglobin. No values were given for oxygen levels of the blood leaving delivery sites in this species. Even if 100% of this oxygen were delivered to the tissues, a stroke volume of 5.23 ml (at the measured heart rate of 7 bts/min) would be required to account for the observed rates of oxygen consumption (Taylor and Brand, 1975a). Since an animal of 10 gm dry body weight cannot have a stroke volume that approaches 5.23 ml, the blood must fuel only a small fraction of aerobic metabolism.

This question of the respiratory role of blood is complicated by several distinctive features of the lamellibranch circulatory system: 1) The blood is oxygenated in the mantle, as well as the gill. 2) Several shunts allow blood to return to the heart without passing through the gill, and the blood may pass through several potential target organs before returning to a site of oxygen uptake. 3) The absence of extensive capillary networks suggests that the transport of oxygen to individual cells must be relatively inefficient. In addition, the partitioning of total oxygen uptake between direct (diffusion from water in superficial tissues) and indirect (diffusion into blood and convection to deep tissues) paths to the tissues is not well known. Thus, there is an incomplete understanding of the respiratory mechanisms which have allowed the majority of lamellibranchs to attain such success with the seeming disadvantage of no respiratory pigment.

In this study, the uptake and transport of oxygen have been exam-

ined in the Atlantic ribbed mussel, Modiolus demissus demissus (Dillwyn) (Order Anisomaria), which has no respiratory pigment in its blood. Since M. demissus inhabits the upper intertidal zone, its respiratory performance was studied in both water and air.

MATERIALS AND METHODS

Mussels were collected from mudflats along the York River estuary (14-18 o/oo salinity) in Virginia, and maintained in air-saturated, recirculating natural water at room temperature (20-23°C).

OXYGEN CONSUMPTION

Mussels were kept in the laboratory for 2-3 weeks prior to measurement of oxygen consumption. Each animal was first scrubbed to remove epiphytes, then placed in a respiratory vessel, and a polarographic oxygen electrode inserted in the top to seal it from the atmosphere. Air-saturated water at room temperature was siphoned through the vessel long enough to allow the mussel to open its valves and ventilate for 20-30 minutes. The siphon hoses were then clamped, stopping the water flow. The depletion of oxygen in the vessel was monitored with a YSI Model 54 O_2 meter and Model 50 laboratory recorder. The experiment was ended when the recorder trace showed no measureable oxygen uptake. The mussel was then removed from its shell and dried at 60°C to a constant weight.

Oxygen consumption in air was measured by placing mussels in custom-made pyrex vessels adapted for a Warburg constant volume respirometer. The vessels were incubated in a water bath at 25°C for at least one hour, or until the mussels opened their valves to air-gape. Oxygen uptake was then measured over a period of 3-4 hours.

To estimate the role of the blood in respiration, oxygen consump-

tion rates were also determined before and after ligation of the anterior aorta. One valve was removed and a thread placed loosely around the aorta. The mussel was then placed in Millipore-filtered water to which streptomycin (0.1%) and penicillin (0.1%) had been added to prevent bacterial growth. Oxygen consumption was first measured for 30 minutes and then the ligature tied off and the measurement repeated. To verify that circulation was blocked by the ligature, a solution of Prussian blue was injected into the hearts of mussels treated in this way. Although the heart continued to contract, the dye did not flow into other regions of the circulatory system.

OXYGEN EXTRACTION

The PO_2 of the exhalant water current was measured with a hypodermic microelectrode (Beckman), whose signal was amplified by a Beckman Model 160 physiological gas analyzer and recorded on a Linear Instr. Corp. Model 112 pen recorder. The microelectrode was lowered with a micromanipulator into the exhalant siphon of mussels burrowed in sand. The PO_2 of the inhalant current was measured with a polarographic oxygen electrode placed near the animal. The ambient PO_2 was gradually lowered in 6-8 steps at intervals of about 30 minutes by bubbling nitrogen gas through the water. Mussels were allowed to ventilate for 10-15 minutes at each new PO_2 before measurements were made. Results were computed by estimating the area under a continuous trace of PO_2 as a function of time (Mangum and Burnett, 1975).

HEART RATE

Platinum pin electrodes were inserted into holes drilled through each valve on either side of the heart, and held in place with dental

wax (Surgident). Heart rates were recorded with an impedance pneumograph and multi-channel pen recorder (E. and M. Instr. Co.). The oxygen level in the water was gradually reduced over a period of 2-3 hours by introducing nitrogen gas.

Heart rates were also recorded in mussels exposed to air. A low tide was simulated by slowly (1.5 hr) siphoning the water from an aquarium containing a mussel burrowed in sand. The mussels were exposed for periods of up to 4.5 hours, after which the water level was slowly (1.5 hr) raised and heart rates monitored for about one hour after full reimmersion.

VENTILATION RATE

Water flow rates from the exhalant siphon of submerged mussels were measured with a thermister flowmeter (LaBarbera and Vogel, 1976) and recorded on a Linear Instr. Corp. Model 112 pen recorder. Voltage to heat the thermister bead was provided by a Staco Incorp. Model E8000 power source. The flowmeter, which senses only current velocity, was calibrated to measure flow rate by pumping water at various rates (determined with a volumetric flowmeter; Emerson Electric Co.) through a tube whose shape and size approximated the mussel's exhalant aperture, past the probe tip. Calibration was performed before and after each experiment and corrections were made for any changes in the probe's response. The ambient PO_2 was gradually lowered in 3-4 steps over a period of 2 hours by introducing nitrogen gas. Ventilation rates were recorded for 4-5 minutes at each PO_2.

GILL SURFACE AREA

Gill dimensions were determined by removing a gill and positioning it on filter paper in its natural shape and tracing its out-

line. The dimensions of individual filaments of a submerged gill were measured under a compound microscope fitted with an ocular micrometer. The gills and remaining body tissue were then dried separately at 60°C to a constant weight.

BLOOD VOLUME

Blood volume was assumed to be equal to the difference in weight between cell water and total water content of the animal. The total wet weight (blood and wet tissue) was measured after prying open the valves to drain the mantle cavity and removing the animal from its shell. After slashing the tissues and allowing the blood to drain for one hour, the tissue was blotted and wet tissue weight obtained. The tissue and blood were then dried separately at 60°C to a constant weight. The cell water content was determined to be the difference between the wet and dry tissue weights. The total water content of the animal was calculated as the difference between total wet weight and the dry weight of the tissue and blood.

BLOOD OXYGEN CARRYING CAPACITY, PO_2, AND pH

The oxygen carrying capacity of the blood was determined with a Lexington Instr. Corp. blood oxygen analyzer (LEX O_2 CON-TL).

Blood samples were obtained anaerobically from various locations in the animal using a 1 ml glass syringe and 25 gauge needle. Approximately 150 µl of blood could be obtained from the heart, while much larger volumes (300-700 µl) were taken from the posterior adductor muscle and mesosomal sinus. Paired samples were taken from two locations within 20 seconds after removing a mussel from the water. Blood PO_2 was measured by injecting samples into a Radiometer Corp.

BMS1 blood gas analyzer. Since a blood with little protein may not layer properly in a liquid-junction electrode, blood pH was determined with a Fisher combination electrode (glass type) and Model 540 digital pH meter.

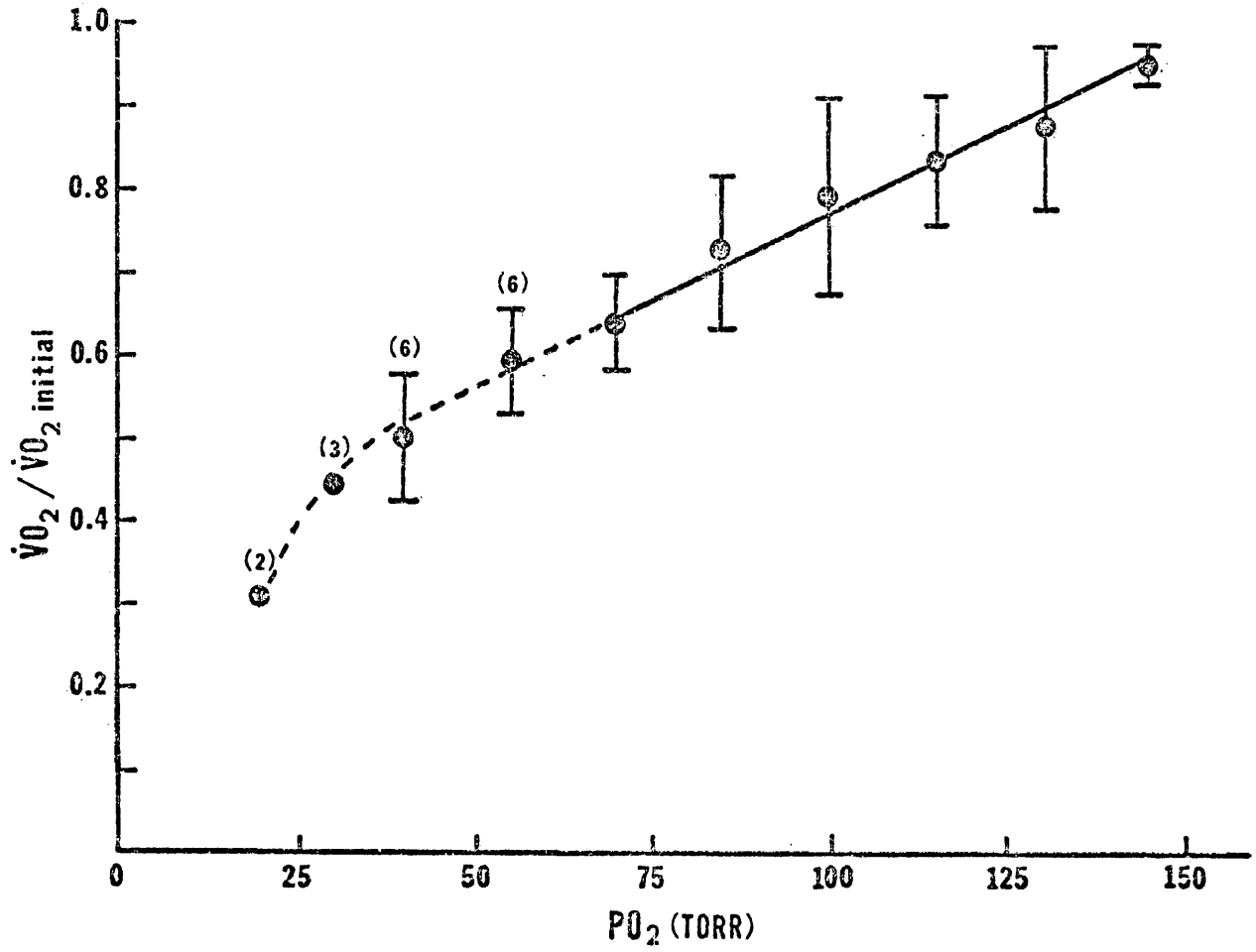
RESULTS

The curve describing normalized $\dot{V}O_2$ as a function of ambient PO_2 (Fig. 1) falls within the range of responses predicted for Modiolus demissus by the quadratic polynomial model of Mangum and Van Winkle (1973), indicating little regulation of oxygen uptake. Paired measurements on mussels after 2 and 5 weeks in the laboratory showed no discernible changes in this pattern, unlike the related species Mytilus edulis (Bayne, 1971).

Modiolus demissus does not exhaust the available oxygen supply but stops consuming oxygen from the water rather suddenly, at a PO_2 between 15 and 45 torr. This response, which also differs from that in M. edulis, is correlated with a higher enzyme activity in the direction of anaerobic pathways than aerobic pathways (Hammen, 1969). Mytilus edulis, which has a lower capacity for anaerobic metabolism (Hammen, 1969), does not stop oxygen uptake while oxygen is still present in the water (Bayne and Livingstone, 1976).

Although M. demissus usually closes its valves when oxygen consumption stops at low PO_2, on several occasions they reopened and gaped widely with the mantle margin spread, as if ventilating. Regardless, no measureable oxygen consumption was detected. Wijsman (1975) noted a similar behavior in M. edulis and attributed it to a periodic flushing of CO_2 produced when the acidic end-products of anaerobic metabolism dissolve the calcareous shell.

Fig. 1. Oxygen consumption by Modiolus demissus in water (20-23°C) at various PO_2's. Initial $\dot{V}O_2$ (μl O_2/gm-hr) values at high PO_2 were set equal to 1.0 and all subsequent values expressed as fractions of 1.0. Points on the solid line represent the mean ($\pm$ S.E.) for seven mussels. Since oxygen uptake ceases at a low, but variable PO_2, points on the dashed line represent mean values for a smaller number of animals, indicated in parentheses.


1.0
0.8
0.6
0.4
0.2
$\dot{V}O_2 / \dot{V}O_2$ initial
(2)
(3)
(6)
(6)
0
25
50
75
100
125
150
PO_2 (TORR)

The mean $\dot{V}O_2$ (± S.E.) for 7 mussels (mean dry weight = 1.35 gm) in water at a PO_2 of 140 torr is 473 ± 16 µl O_2/gm-hr (N = 14). In air, $\dot{V}O_2$ is more variable, reflecting the extent of shell gape; mean $\dot{V}O_2$ (± S.E.) for 6 mussels (mean dry weight = 2.43 gm) is 226 ± 21 µl O_2/gm-hr (N = 19). The latter value is very close to that found under similar conditions by Kuenzler (1961). Using his estimates of the effect of body size on aerial $\dot{V}O_2$, the rate in a 1.35 gm animal should be 232 µl O_2/gm-hr. Thus, $\dot{V}O_2$ in air is reduced to about 50% of that in water, which is a considerably larger decrease than found by Kuenzler (1961). The ratio of aerial to aquatic $\dot{V}O_2$ (0.49) is also low in comparison with Mytilus californianus (0.738) and Cardium edule (0.655) (Bayne, et al, 1976b) and may be explained by the preferance for anaerobic metabolism (Hammen, 1969). However, these results may even underestimate the difference; aquatic oxygen uptake was determined in November, when the gonads may be depleted of gametes, and aerial oxygen uptake in July, when they are ripe. If aerial $\dot{V}O_2$ increases during gametogenesis, as does aquatic $\dot{V}O_2$ (Bayne, 1973), the ratio in M. demissus may be even lower.

The removal of one valve does not alter oxygen consumption rates; the rates are within the range found in intact animals. More importantly, ligation of the anterior aorta decreases oxygen uptake only by about 10-15% (Table 1).

OXYGEN EXTRACTION

Oxygen extraction from the ventilatory current (Fig. 2) increases from 7-8% near air saturation to about 35% at a PO_2 of 25-30 torr, a compensatory response which is also found in several other lamellibranchs (Mangum and Burnett, 1975; Taylor and Brand, 1975b).

TABLE 1

THE EFFECTS OF LIGATION OF THE ANTERIOR AORTA ON OXYGEN CONSUMPTION IN *MODIOLUS DEMISSUS*, 21°C, AMBIENT PO_2 = 132-150 TORR.

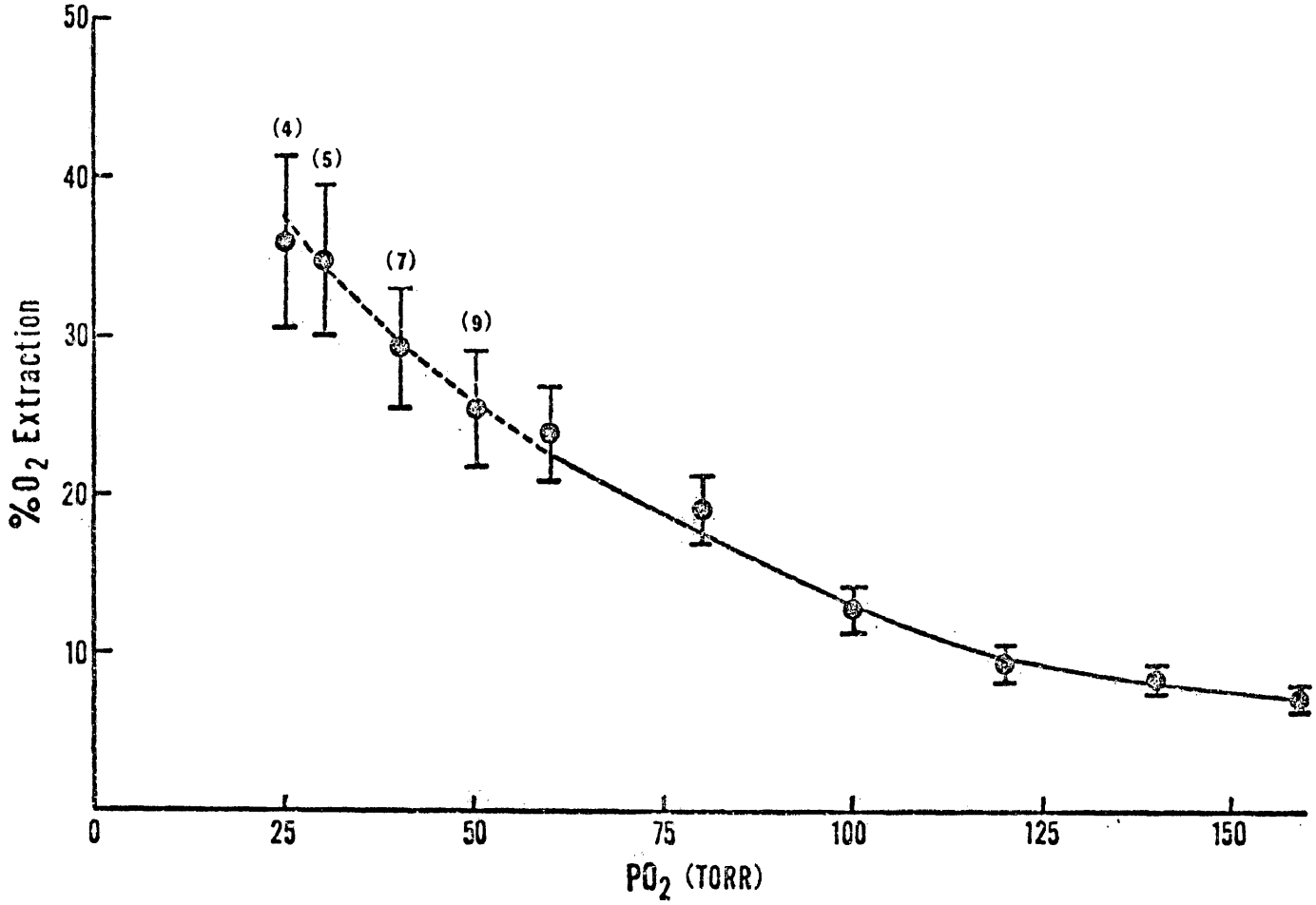
A. Paired measurements

	Control	Ligated	
Mussel	$\dot{V}O_2$ (µl O_2/g-hr)	$\dot{V}O_2$ (µl O_2/g-hr)	%Change
1	518.97	534.24	+ 2.9%
2	448.41	398.59	- 11.1%
3	448.91	356.49	- 20.6%

B. Unpaired measurements (3 mussels unligated, 3 mussels ligated)

Control	Ligated	
Mean $\dot{V}O_2$ (µl O_2/g-hr)	Mean $\dot{V}O_2$ (µl O_2/g-hr)	%Change
444 ± 31 (S.E.)(N=6)	377 ± 43 (S.E.)(N=6)	- 15.1%

Fig. 2. Per cent of oxygen extracted from the water (20-23°C) by Modiolus demissus at various ambient oxygen levels. Points on the solid line are the mean (± S.E.) for ten mussels, and those on the dashed line represent the mean (± S.E.) for a smaller number of animals (indicated in parentheses) in which oxygen uptake persisted at low PO_2.


50
40
30
20
10
%O2 Extraction
(4)
(5)
(7)
(9)
0
25
50
75
100
125
150
PO2 (TORR)

HEART RATE

At high PO_2, heart rates generally ranged from 12-16 bt/min, although baseline rates as low as 7 bt/min and as high as 17 bt/min were recorded. Since baseline rates were so variable even in a single animal, the values were normalized for presentation (Fig. 3). The most common response to declining oxygen is shown by mussels A, B, and D (Fig. 3); an increase in rate (2-4 bt/min or 12-22%) is followed by a sharp decrease at low PO_2, immediately before valve closure. The heart rate in one animal (C) slowed gradually until oxygen uptake ceased. Shortly after valve closure, heart rate drops to about 3-5 bt/min. The phenomenon of complete cardiac arrest found in Mytilus edulis (Bayne, 1971), never occurred in M. demissus, even after the valves had been closed for several hours. Otherwise, the typical response in M. demissus resembles that in other lamellibranchs, eg. M. edulis (Bayne, 1971), Pecten maximus (Brand and Roberts, 1973), and Arctica islandica (Taylor and Brand, 1975b), despite the absence of aerobic shutdown in these species.

Exposure to air for periods of up to 4.5 hours caused no significant changes in heart rate in 5 sets of paired observations ($P > 0.05$; student's t-test). A typical response, in which heart rate remains constant at 16 bt/min is shown in Fig. 4 (A and B). The persistence of circulation in M. demissus is strikingly different from the responses in several other air-gaping lamellibranchs. With two exceptions (Trueman and Lowe, 1971; Boyden, 1972), the intertidal and subtidal species studied previously show considerable bradycardia, or even complete suppression of heart rate, usually within one hour after becoming exposed to air (Helm and Trueman, 1967; Coleman and

Fig. 3. The effects of reduced ambient PO_2 on heart rate in four submerged mussels (20-23°C). Initial heart rates (beats per minute) at high PO_2 expressed as 1.0, and all subsequent heart rates as fractions of 1.0.

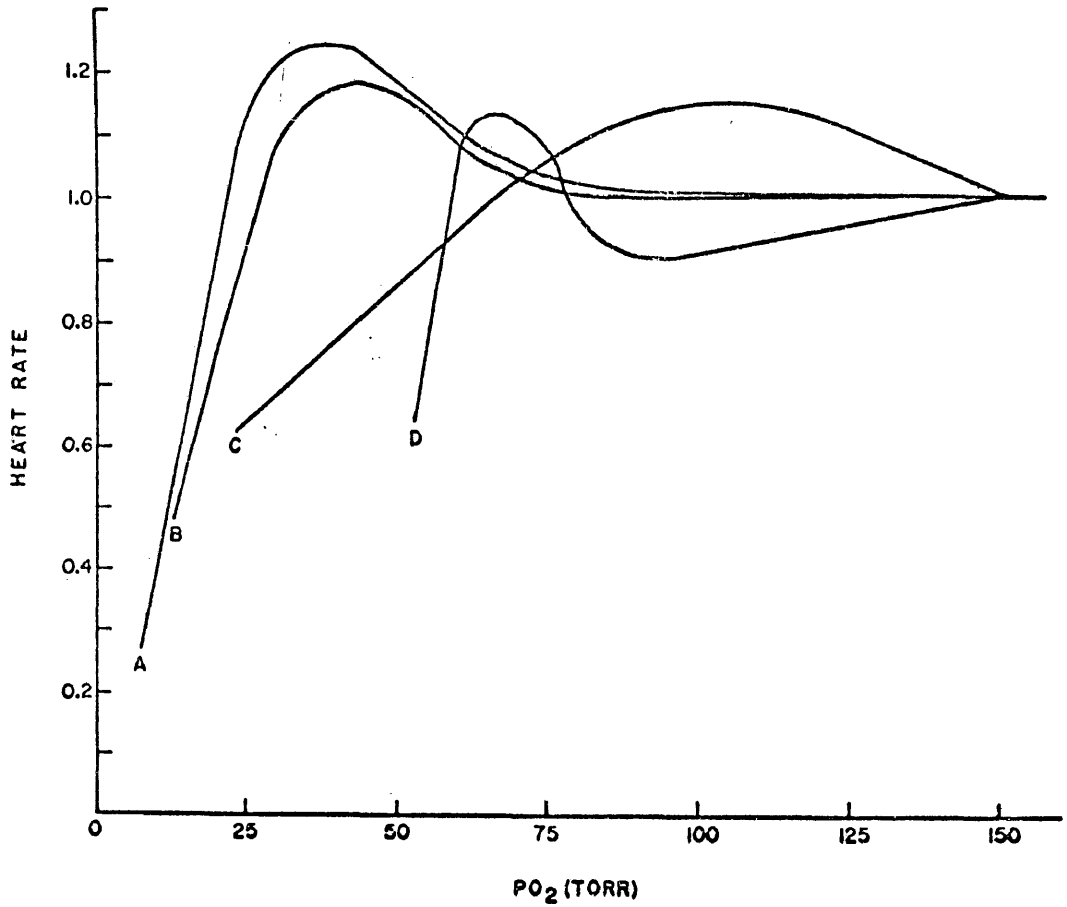
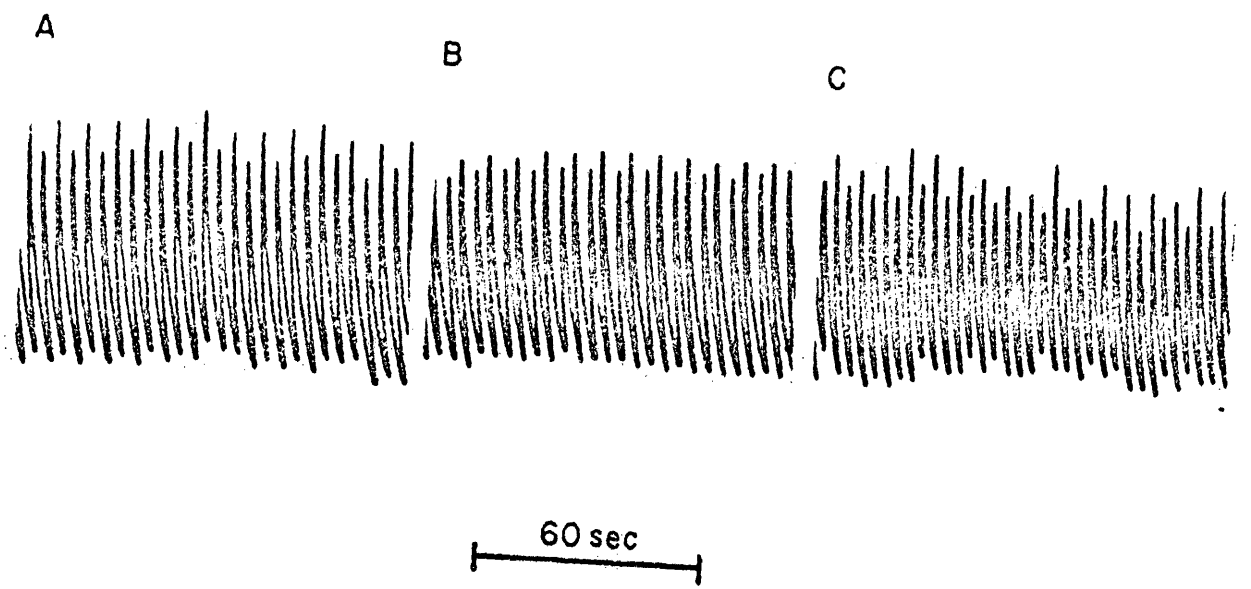

HEART RATE
1.2
1.0
0.8
0.6
0.4
0.2
0
25
50
75
100
125
150
A
B
C
D
PO2 (TORR)

Fig. 4. Heart rate in Modiolus demissus during air exposure (21°C).
A = 16 bt/min, prior to exposure (water PO_2 = 150 torr).
B = 16 bt/min, exposed to air for 70 minutes.
C = 18 bt/min, immediately following reimmersion (water PO_2 = 150 torr).


A
B
C
60 sec

Trueman, 1971; Coleman, 1973; Brand and Roberts, 1973; Coleman, 1976; Bayne, et al, 1976b).

The capacity of lamellibranchs to buffer organic acids produced during anaerobic metabolism in air would be increased by release of the CO_2 formed when the shell is dissolved; therefore, de Zwaan and Wijsman (1976) propose that air-gaping may serve to eliminate CO_2 rather than to maintain aerobic metabolism. In M. demissus, the release of excess CO_2 from the blood may be enhanced by the maintenance of a normal heart rate during air exposure. The advantage of eliminating CO_2 must be balanced against the energetic cost of a relatively high heart rate. Thus, short bursts of cardiac activity observed periodically in several other air-gaping species (Coleman and Trueman, 1971; Brand and Roberts, 1973) may serve the same function, though perhaps less efficiently.

Upon reimmersion, heart rates in three of five mussels increased briefly by 2-4 bt/min (15-25%), while two showed no change. An example of an increase in rate from 16 to 18 bt/min following air exposure is shown in Fig. 4C. In the cases of increase, the rates returned to control levels within one hour. This increase in circulation, which must entail increased energy expenditure, is a response which may explain the frequently observed "oxygen debt" phenomenon in species that do not oxidize the accumulated end-products of anaerobic metabolism (Mangum and Burnett, 1975). It may simply serve to flush anaerobically produced acids from the animal.

The amplitude of the heart beat as indicated by the deflection of the recording pen was quite variable, showing no correlation with heart rate. Therefore, an estimate of cardiac output from the product

of heart rate and amplitude (Bayne, 1971; Taylor and Brand, 1975b) would be highly inaccurate, and thus was not attempted.

VENTILATION RATE

Ventilation rates in Modiolus demissus generally decrease at low PO_2's (Fig. 5), and thus less oxygen is made available to the animal. The measured ventilation rates are slightly higher than those predicted from the Fick equation, using observed values of oxygen consumption and oxygen extraction. The small discrepancy is probably due to the procedure of volumetrically calibrating the flowmeter. A tube approximating the maximum dimensions of the exhalant siphon was used, whereas the mussels continually change the shape of their exhalant aperture. Reductions in the size of the siphonal aperture could increase the exhalant current velocity without increasing the volumetric flow rate, which would result in the measured rates being overestimated.

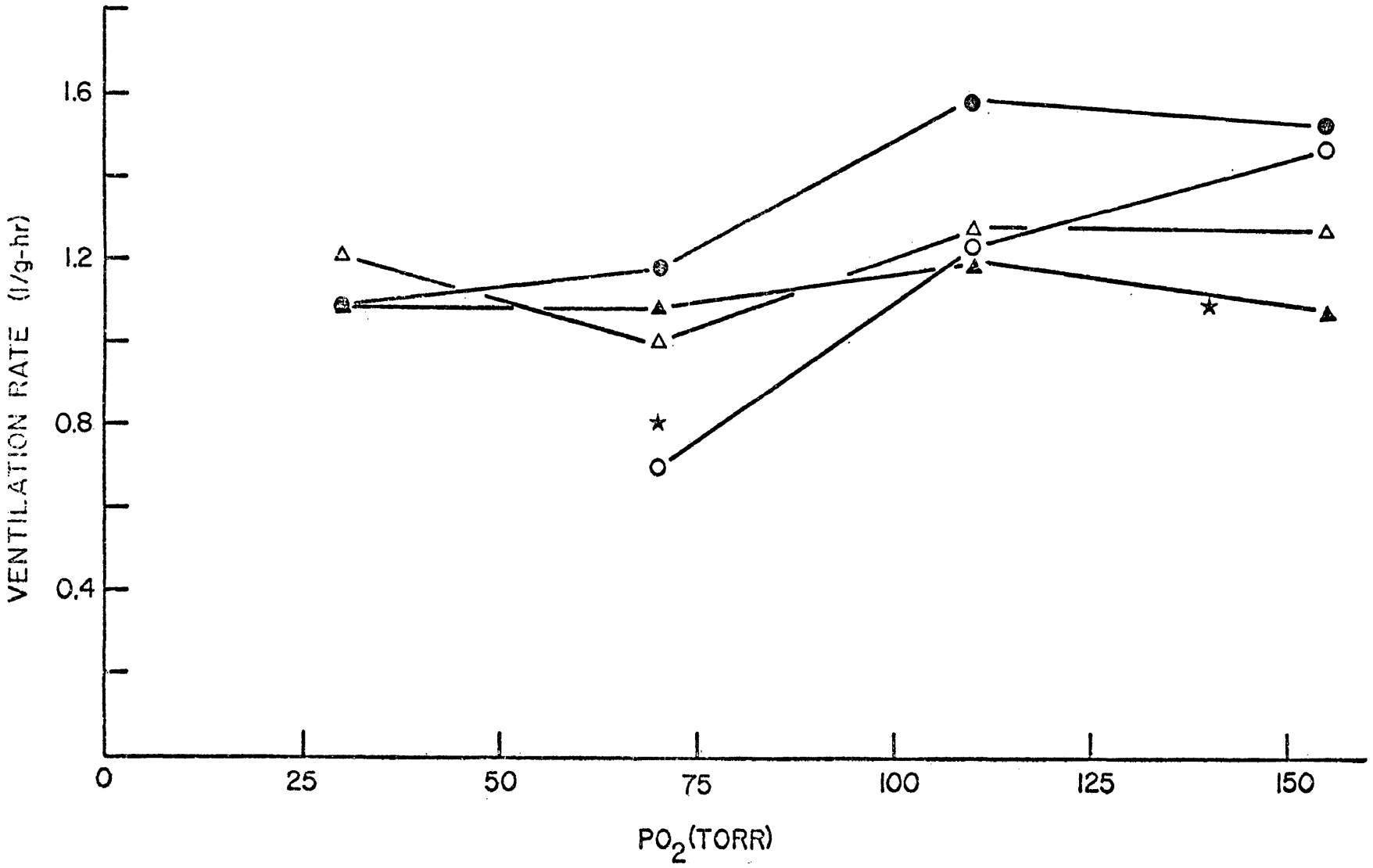
GILL SURFACE AREA

In 6 mussels (1.15-3.87 gm dry weight), the dry weight of the gills comprises 8.2% (± 0.68 S.E.) of total dry body weight. The shape of a demibranch approximates a trapezoid measuring 5.20 X 1.48 X 3.20 X 1.48 cm in a 20.15 gm wet weight (1.35 gm dry weight) mussel. There are about 950 filaments and, in each lamella, a filament measures 0.0041 cm in width (frontal surface) and 0.0186 cm in depth (lateral surface). The total surface area for gas exchange is approximately 13.9 cm^2/gm animal wet weight, which is close to the value of 13.5 cm^2/gm for Mytilus (Ghiretti, 1966).

BLOOD VOLUME

The total water content in 10 mussels (mean wet weight = 20.4 gm

Fig. 5. Ventilation rates of four mussels in declining ambient oxygen (22-23°C). ★ = indicates ventilation rate predicted from experimental values for oxygen consumption and oxygen extraction.


VENTILATION RATE (l/g-hr)
1.6
1.2
0.8
0.4
0
25
50
75
100
125
150
PO2(TORR)

± 1.67 S.E.) is 93.3% (± 3.52 S.E.) of the total wet weight, or 19.03 gms. Blood accounts for 67.2% of the total water content. Thus, the blood volume is approximately 12.8 ml, or 62.7% of total wet weight. This latter value is close to that for Noetia ponderosa (60.2%; Deaton and Mangum, 1976), however, it is somewhat higher than the blood volume of Mytilus californianus (50.8%) and Margaritana margaritifera (49.0%) (Martin, et al, 1958).

BLOOD OXYGEN CARRYING CAPACITY, PO_2, AND pH

The oxygen concentration of blood equilibrated in vitro to the atmosphere (0.6 vols%) does not differ from that of water at the same salinity and temperature. There is no evidence of a specialized oxygen carrier in the blood.

A generalized diagram of the circulatory system in Modiolus demissus, based on an account of mytilid anatomy by White (1937) and personal observations is shown in Fig. 6. From the longitudinal vein (LV), the heart receives both oxygenated blood from the gills (G) and deoxygenated blood from the viscera (Vi). Therefore, mixing of the blood should result in a PO_2 in the heart which is lower than that of blood leaving the gills. The efferent branchial vessel is too small to be sampled anaerobically; however, the extent to which the blood can be oxygenated should be closely approximated by the highest PO_2's observed, which are found in blood from the mesosomal sinus (Table 2A). The values found in the mesosomal and intermuscular sinuses (shown together as superficial sinuses, SS, in Fig. 6) are high and similar to one another, suggesting that these sinuses are major sites of oxygen uptake by the blood, along with the gill, and probably the mantle. Because oxygen uptake occurs at several sites with so much

Fig. 6. Major circulatory routes in Modiolus demissus showing blood PO_2's at various points. PM = posterior adductor muscle, AM = anterior adductor muscle, LV = longitudinal vein, SS = superficial sinuses (mesosoma and intermuscular sinuses), K = kidney, Ve = ventricle, A = auricle, G = gill, Vi = viscera, F = foot, M = mantle, ▒▒ = oxygenated blood, ═══ = deoxygenated blood, ||| = mixed blood.

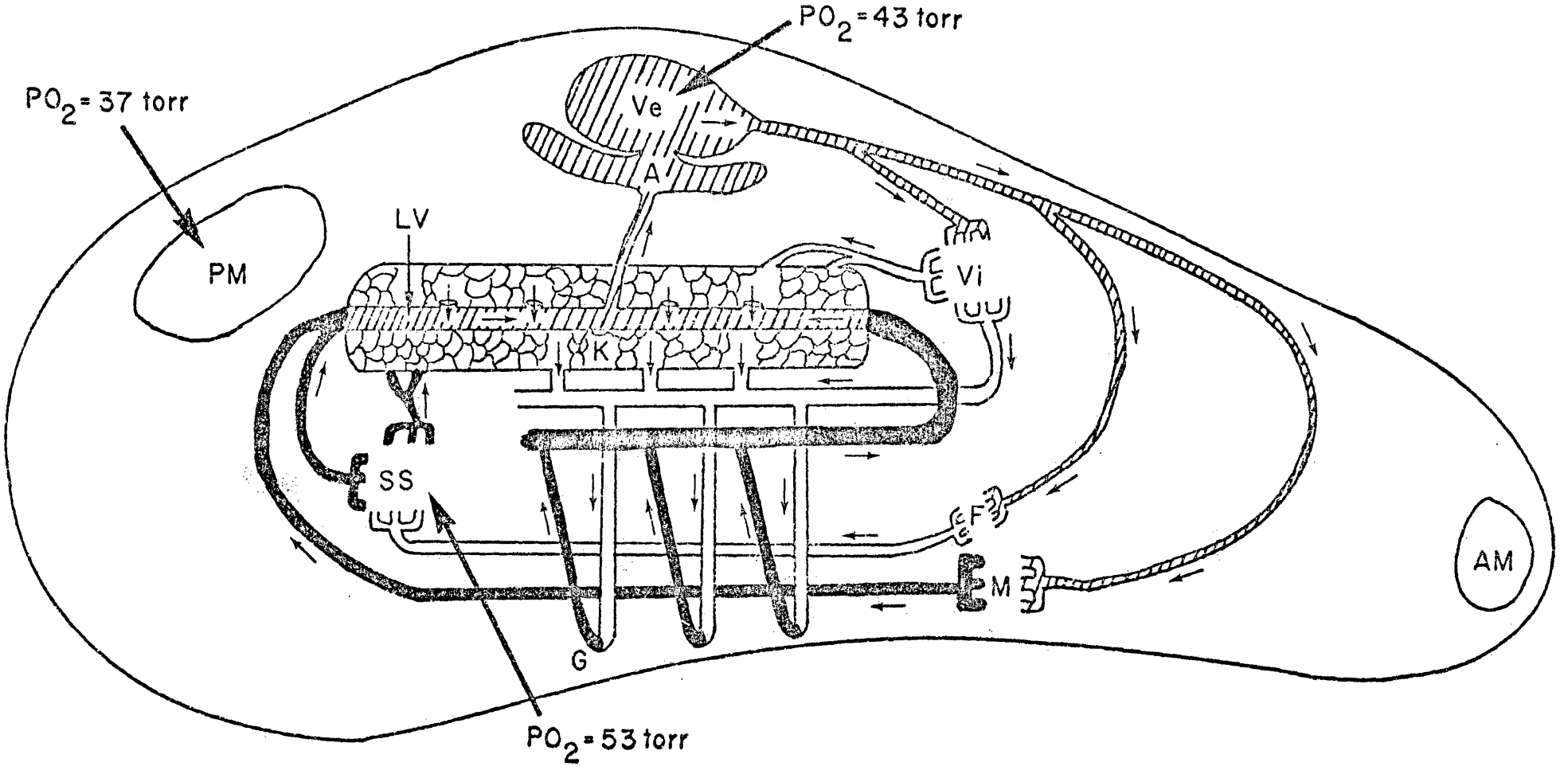

PO_2 = 43 torr
PO_2 = 37 torr
Ve
A
LV
PM
Vi
K
SS
F
M
AM
G
PO_2 = 53 torr

TABLE 2

BLOOD OXYGEN LEVELS IN *MODIOLUS DEMISSUS* (MEAN ± S.E.; NUMBER OF OBSERVATIONS INDICATED IN PARENTHESES)

	PO_2 (Torr)			
Ambient	Mantle Cavity	Heart	Post. Adductor Muscle	Mesosoma
A. Immersed in Water				
145-155	111 ± 3.1 (51)	43 ± 8.9 (6)*	37 ± 3.4 (10)	53 ± 9 (6)*
68-74	---	---	28 ± 1.7 (9)	---
64-68	---	31 ± 3.1 (6)*	23 ± 2.6 (6)*	---
60-64	---	---	---	22 ± 2.7 (6)
B. Exposed to Air (Air-gaping)				
Time Exposed (Hr)				
1	---	45 ± 4.9 (5)*	---	55 ± 6.7 (5)*
6	32 ± 4.4 (5)*	29 ± 3.5 (5)*	26 ± 3.3 (5)*	---

* = paired samples

mixing of efferent and afferent blood, the conventional terms pre- and postbranchial do not reflect the oxygenation state of the blood.

Both the mesosoma and intermuscular sinus appear externally as relatively thin-walled, blood-filled sacs along the ventral edge of the visceral mass, where they expose a large surface area to the inhalant current. The thickness of the membrane separating water from the blood in the mesosoma is not known. However, the sinus is often highly inflated, distending the wall and thus decreasing the diffusion barrier. Although a relatively small diffusion distance separates the mesosomal blood from water, mesosomal blood also bathes the byssus retractor muscles and must deliver some of its oxygen to these tissues. Blood from the mesosoma empties into either the intermuscular sinus or the longitudinal vein (White, 1937). The intermuscular sinus receives blood from the mesosoma and foot which then empties into the kidney (White, 1937). Thus, the blood in the superficial sinuses is a mixture of oxygenated and deoxygenated blood. Yet, the PO_2 of blood in the mesosoma (53 torr) is comparable to that in the heart of the hemoglobin-containing clams Noetia ponderosa (PO_2 = 62 torr; Freadman and Mangum, 1976) and Anadara ovalis (PO_2 = 55 torr; Mangum, 1973) at the same temperature. However, the PO_2 of blood in a deep sinus, such as that in the posterior adductor muscle remains high (Table 2A), indicating a low rate of extraction of oxygen (14%) from the blood.

When the water PO_2 is lowered to 60-68 torr, PO_2 in the heart and posterior adductor muscle both drop (Table 2A), but the per cent extraction of oxygen from the blood almost doubles. At the same time, mesosomal PO_2 is reduced to a value slightly lower than that of

the heart.

Air exposure for one hour has no effect on blood PO_2 (Table 2B). However, within six hours, the values drop substantially. During this time, the PO_2 of the mantle cavity fluid is reduced to about 32 torr. Paired samples show a PO_2 difference of only 3 torr between mantle cavity fluid and the heart, and between the heart and posterior adductor muscle (Table 2B). Under these conditions, aerobic metabolism must be confined primarily to superficial tissues in adequate contact with the air, while anaerobic metabolism must become more important in deep tissues. This conclusion is supported by the substantial reduction in $\dot{V}O_2$ during air exposure (see above).

Blood PO_2 decreases in air exposed mussels whose valves have been tightly clamped. Mesosomal PO_2 (Fig. 7) decreases sharply over the first two hours, but does not drop below 11 torr, even after 3.5 hours of valve closure. A similar drop in PO_2 occurs in blood from the posterior adductor muscle (Fig. 7). This result suggests that aerobic respiration continues immediately after valve closure utilizing oxygen from the blood, although it shuts down completely when blood PO_2 reaches 10-15 torr. Taylor (1976a) found measureable oxygen levels in the mantle cavity fluid and blood of several individuals of Arctica islandica which had been under anoxic conditions for five days. However, the PO_2 of mantle cavity fluid in Mytilus californianus is reduced to zero within one hour after valve closure (Moon and Pritchard, 1970).

After 24 hours without oxygen, which M. demissus easily tolerates (Lent, 1968), there is a pH drop of 0.48 both in blood from the posterior adductor muscle and in mantle cavity fluid (Table 3A), although

Fig. 7. Blood PO_2 in Modiolus demissus during anaerobiosis (21°C).
● = mesosoma, O = posterior adductor muscle.

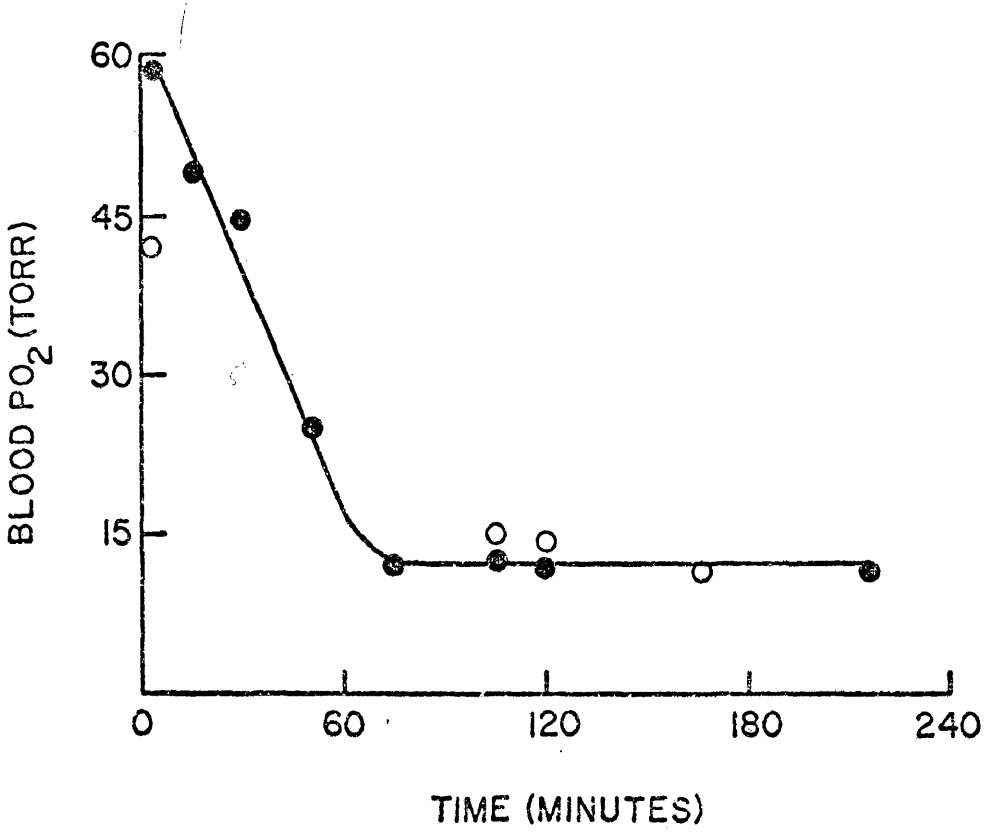

60
45
30
15
BLOOD PO_2 (TORR)
0
60
120
180
240
TIME (MINUTES)

TABLE 3

BLOOD AND MANTLE CAVITY FLUID pH IN *MODIOLUS DEMISSUS* EXPOSED TO AIR (MEAN ± S.E.), 22°C.

	Time Exposed (Hr)		
	0	6	24
A. Anaerobiosis (valves clamped) (N=4)			
Post. Adductor	7.27 ± 0.040	7.09 ± 0.176	6.79 ± 0.137
Mantle Cavity	7.05 ± 0.042	6.82 ± 0.117	6.57 ± 0.167
B. Air-gaping (N=4)			
Post. Adductor	7.28 ± 0.139	---	7.02 ± 0.028
Mantle Cavity	6.72 ± 0.070	---	6.74 ± 0.043

the mantle cavity fluid is always more acid than the blood. Mytilus edulis shows changes in pH of 0.5-0.9 during 24 hours of continuous valve closure (Wijsman, 1975).

When M. demissus is allowed to air gape for 24 hours, blood pH drops only by 0.26, while the pH of mantle cavity fluid does not change significantly (Table 3B). This might be attributed to a lower concentration of acid end-products from anerobic metabolism during air-gaping, however, it is more likely a result of the release of CO_2 produced when these acids are buffered by the shell (Wijsman, 1975).

During periods of anaerobiosis, blood pH in M. demissus is above the pH optimum (6.5-6.6) for the enzyme phosphoenolpyruvate carboxykinase (de Zwaan and de Bont, 1975), which directs the flow of carbon into anaerobic pathways. This suggests that aerobic pathways predominate. However, the results of in vivo and in vitro studies are contradictory; under hypoxic conditions, substances believed to be anaerobic end-products accumulate in the posterior adductor muscle of Mytilus edulis (de Zwaan and Zandee, 1972; Livingstone and Bayne, 1976). Yet, Wijsman (1975) found that, even under anoxic conditions, submerged M. edulis maintains its pH at 7.3-7.5 by periodic flushing of the mantle cavity. In M. demissus, the persistence of oxygen in the blood after several hours of valve closure suggests that anaerobic respiration predominates, despite the relatively high blood pH.

DISCUSSION

While gas exchange across permeable body surfaces must occur to some degree in all aquatic molluscs, it is often overlooked. The large amounts of soft body tissue exposed to the ventilatory current suggest that the direct entry of oxygen into these tissues may account for a substantial fraction of total oxygen uptake in the lamellibranchs. In addition, the high mesosomal PO_2's in Modiolus demissus indicate that branchial and pallial oxygen uptake in the mytilid body type are further supplemented by the large superficial sinuses. Thus, the sites of gas exchange are far more numerous than portrayed previously, resulting in high blood PO_2's which are in the range found in species with respiratory pigments.

However, the small decrease in $\dot{V}O_2$ after blocking circulation, and the very small reduction of blood PO_2 in deep tissues when circulation is unimpaired, indicate that the blood plays a limited role in transporting oxygen. A greater delivery of oxygen to the tissues in the posterior adductor muscle has been found in Mytilus edulis (B. L. Bayne, personal communication). However, the present author has found that PO_2 of blood from the adductor muscle is also high (40 torr) in Mya arenaria, suggesting that the results for M. demissus are not atypical. In addition, lamellibranchs are very tolerant of blood loss, and the survival of Mytilus californianus is unaffected for several weeks after most of its blood is withdrawn (C. J. Costa, personal communication). Thus, the respiratory function of the blood in these

lamellibranchs must be small, and the circulating body fluids must be primarily involved in other physiological functions such as nutrition, salt and water balance, and excretion of metabolic wastes. This conclusion is supported by the unrealistic estimate obtained for blood flow by computations, assuming that blood is an important carrier of oxygen. For example, if 1) the blood transports 100% of the oxygen consumed, and 2) the highest (mesosoma) and lowest (posterior adductor) PO_2's observed are actually representative of all sites of uptake and delivery, then the Fick equation predicts that cardiac output of a 1.35 gm animal would be 17.7 ml/min. At a heart rate of 14 bt/min, stroke volume would be 1.26 ml, whereas the actual heart volume, estimated by syringe withdrawl and distension, is only 0.150-0.175 ml. If, however, the blood carries only a small fraction (10-15%) of the oxygen consumed, which was inferred from the experimental blockage of circulation, stroke volume would only be 0.126-0.189 ml.

Even though blood-borne oxygen accounts for a relatively small portion of total oxygen uptake, the blood may still be essential in transporting oxygen to specific tissues such as the adductor muscles, which comprise a small fraction of the total biomass. Nonetheless, it is possible that some deep tissues may always function anaerobically, regardless of external oxygen levels. Mitochondria are not very abundant in adductor muscle tissue (Mattisson and Beechey, 1966; Hochachka and Mustafa, 1973) and they may be relatively small compaired to those in more active muscles (Hanson and Lowy, 1961). Tappel (1960) found low concentrations of cytochromes in the adductors of several lamellibranchs. Likewise, Addink and Veenhof (1975) found high activities for glycolytic enzymes in the posterior adductor

muscle of Mytilus edulis, suggesting that its primary source of energy is glycolysis. However, while the activity of anaerobic pathways in the adductor muscle may increase at low oxygen, the blood must supply aerobic pathways as well, as indicated by the continuation of oxygen removal from the blood at low ambient PO_2. The increase in extraction of oxygen from the blood reflects a greater importance of blood under hypoxic conditions. However, the decrease in ventilation rate at lower PO_2's is not offset by the small increase in the respiratory role of blood, and oxygen consumption is not maintained at a constant level.

When M. demissus air-gapes, the virtual equilibration of PO_2's in the mantle cavity fluid and the blood, and the small extraction of oxygen from the blood in the posterior adductor muscle suggest that the tissues consume very little oxygen from the blood. This is consistent with the results of Bayne, et al (1976b), who found an accumulation of anaerobic end-products in the adductor muscle of M. californianus during air-gaping. In addition, Coleman (1973) found that slitting the posterior adductor muscle, which must result in blood loss, does not significantly alter oxygen consumption in M. edulis during air-gaping. The major portion of oxygen consumed, therefore, is probably extracted by the tissues directly from the mantle cavity water, or, when water is lost, from the air. If the low PO_2 of the mantle cavity fluid is the limiting factor of oxygen uptake during air exposure, then $\dot{V}O_2$ may depend on the amount of water retained in the mantle cavity; a loss of water would expose a greater amount of tissue to the air. The resultant level of aerobic metabolism may be a compromise between the demands of energy produc-

tion and resistance to dessication.

A comparison of respiratory parameters in Modiolus demissus with those of the hemoglobin-containing species Noetia ponderosa, is presented in Table 4. Despite the high oxygen carrying capacity of its blood and the large difference between oxygen concentration of afferent and efferent blood, $\dot{V}O_2$ in N. ponderosa is about 67% of that in M. demissus. Similarly, Krüger (1958) found that $\dot{V}O_2$ in Glycymeris nummaria is greatest in individuals with the lowest hemoglobin concentrations. The loss of a pigment that increases oxygen extraction from the water is accompanied by an increase in ventilation rate. This increase can be brought about 1) by enlarging gill surface area, and 2) by pumping harder, which must increase the metabolic demand. The larger gill surface area in M. demissus (Table 4) is primarily due to the width of the lateral surface of the gill filaments (0.0186 cm) which is more than three times that in N. ponderosa (0.0056 cm; Deaton and Mangum, 1976). The dimensions of the frontal surface of the filaments are similar in the two species. Since the cilia responsible for the ventilatory current are located on the lateral surface of the filaments, the differences in ventilatory rate may be explained by the difference in gill surface area. However, since blood volume and heart rates in N. ponderosa (L. E. Deaton, unpublished data) and M. demissus are similar, the higher oxygen concentration of efferent blood in N. ponderosa suggests that the enlargement of gill surface area cannot compensate for a greatly reduced oxygen carrying capacity of the blood.

The evolution of high pumping rates in lamellibranchs is generally regarded as an adaptation more for filter-feeding than for gas

TABLE 4

RESPIRATORY PARAMETERS OF *MODIOLUS DEMISSUS* AND *NOETIA PONDEROSA*,

AMBIENT PO_2 = 140 TORR, 21-23°C.

Parameter	*M. demissus*	*N. ponderosa* (Freadman and Mangum, 1976; Deaton and Mangum, 1976)
$\dot{V}O_2$	473 µl O_2/gm dry-hr	316 µl O_2/gm dry-hr
Ventilation Rate	1.34 l/gm dry-hr* 1.1 l/gm dry-hr**	0.1 l/gm dry-hr
% Oxygen Extraction	8-9%	60%
Gill Surface Area	13.9 cm^2/gm wet	4.6-5.7 cm^2/gm wet
Blood Oxygen O_2 Carrying Capacity	0.6 vols%	4.53 vols%
Heart PO_2	43 torr	62 torr
Heart O_2 Concentration	0.16 vols%	3.92 vols%
Tissue PO_2	53 torr (mesosoma) 37 torr (post. adductor)	7 torr (hepatic sinus)
Tissue O_2 Concentration	0.20 vols% (mesosoma) 0.14 vols% (post. adductor)	2.10 vols% (hepatic sinus)

* experimentally measured rate - see Fig. 5.

** calculated from oxygen consumption and oxygen extraction - see Fig. 5.

exchange, based on the low oxygen extraction efficiencies in most species. However, the ventilation rate in M. demissus may be related to its rate of aerobic metabolism as well as its nutritional state. The measured per cent oxygen extraction is an average of oxygen removal at many superficial sites, in addition to the gill. Water passing through the mantle cavity may transport oxygen to remote body surfaces which extract it with a high efficiency, while oxygen extraction at the gill is low. If the ventilation rate is increased until the more remote tissue is adequately fueled, the average rate of oxygen extraction by all of the multiple sites might be low. Bayne, et al (1976a) have calculated that the mechanics of ventilation require 59% of the oxygen consumed in Mytilus californianus. Thus, the difference between $\dot{V}O_2$ in M. demissus and N. ponderosa might be at least partially explained by a ventilation rate in M. demissus which is more than ten times that of N. ponderosa.

Regardless, it is apparent that the role of the blood in transporting oxygen differs greatly in species with a functional respiratory pigment and those with none. It seems that, in Modiolus demissus and some other members of the family Mytilidae, blood may become important only under conditions of low oxygen; at high ambient PO_2, their metabolism does not involve the substantial participation of the circulatory system. This shift in the respiratory role of the blood is accompanied by an ability to supply superficial tissues with oxygen derived from air, which, along with the capacity for facultative anaerobiosis in deep tissue, is a major factor in allowing the successful exploitation of the upper intertidal zone.

SUMMARY

1. The blood of Modiolus demissus, which lacks a respiratory pigment, equilibrates with oxygen in the water to a degree comparable to species with blood pigments; however, only 14% of the oxygen is delivered to the tissues.

2. The high blood PO_2 in the mesosoma suggests that this is an important site for blood gas exchange, along with the gill and mantle.

3. In highly oxygenated water, blocking the circulatory system depresses oxygen consumption rates by only 15%, indicating that the primary route of oxygen uptake is direct, into superficial tissues.

4. At low ambient oxygen, there is an increase in heart rate and per cent oxygen extraction from the water; however, M. demissus is a poor regulator of oxygen consumption.

5. When water PO_2 falls below 40-50 torr, M. demissus generally closes its valves and ceases to respire aerobically.

6. During air exposure, M. demissus extracts oxygen from the air, although its rate of oxygen uptake is reduced by 50%. At this time, the primary pathways of metabolism in deep tissue are probably anaerobic.

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